(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 021 723 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
10.12.2008 Bulletin 2008/50

(21) Application number: **98948399.5**

(22) Date of filing: **22.09.1998**

(51) Int Cl.:
G01N 33/576 *(2006.01)* ***G01N 33/53*** *(2006.01)*

(86) International application number:
PCT/US1998/019694

(87) International publication number:
WO 1999/015901 (01.04.1999 Gazette 1999/13)

(54) BUFFERS FOR STABILIZING HCV ANTIGENS

PUFFERN ZUR STABILIZIERUNG VON HCV ANTIGENEN

TAMPONS POUR HCV ANTIGENES DE STABILISATION

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **22.09.1997 US 59703 P**

(43) Date of publication of application:
26.07.2000 Bulletin 2000/30

(73) Proprietor: **Novartis Vaccines and Diagnostics, Inc.**
Emeryville, CA 94608 (US)

(72) Inventors:
- **CHIEN, David, Y.**
  Alamo, CA 94507 (US)
- **ARCANGEL, Phillip**
  Berkeley, CA 94704 (US)
- **TIRELL, Stephen**
  Wellesley, MA 02482-2258 (US)
- **ZIEGLER, Wanda**
  Medway, MA 02032 (US)

(74) Representative: **Woods, Geoffrey Corlett et al**
J.A. KEMP & CO.
14 South Square
Gray's Inn
London
WC1R 5JJ (GB)

(56) References cited:

EP-A- 0 341 439	WO-A-94/25874
DE-A- 19 502 386	US-A- 5 284 932
US-A- 5 616 460	US-A- 5 681 695
US-A- 5 705 330	US-A- 5 773 212

- **PATENT ABSTRACTS OF JAPAN vol. 018, no. 321 (P-1756), 17 June 1994 & JP 06 074956 A (DAINABOTSUTO KK), 18 March 1994**
- **COHARD M ET AL: "Hepatitis C virus-specific CTL responses in PBMC from chimpanzees with chronic hepatitis C: determination of CTL and CTL precursor frequencies using a recombinant canarypox virus (ALVAC)" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 214, no. 1-2, 1 May 1998, page 121-129 XP004129944**
- **IDE Y ET AL: "Hepatitis C virus NS5A protein is phosphorylated in vitro by a stably bound protein kinase from HeLa cells and by cAMP-dependent protein kinase A-alpha catalytic subunit" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, vol. 201, no. 1-2, 12 November 1997, page 151-158 XP004126467**

EP 1 021 723 B1

Printed by Jouve, 75001 PARIS (FR)

Description

[0001] This application claims priority benefit under 35 U.S.C. §119 to Application Serial No. 60/059,703.

FIELD OF THE INVENTION

[0002] The present invention is related generally to the field of immunoassays and specifically to buffers for stabilizing antigens, in particular hepatitis C virus (HCV) antigens, for use in anti-HCV immunoassays.

BACKGROUND OF THE INVENTION

[0003] In general, immunoassays are produced by first determining epitopes that are specifically associated with a virus and then determining which of the epitopes is preferred for the assay being developed. When the particular epitopes are isolated, their sequences are determined, and genetic material for producing the epitopes is produced. Methods of producing proteins by either chemical or biological means are known, as are assays used to detect the presence of antibodies to particular epitopes. Highly selective and sensitive immunoassays generally contain major immunodominant epitopes of the pathogen suspected of infecting a patient.

[0004] For the virus HCV, major immunodominant linear epitopes have been identified from the core, NS3 (nonstructural), NS4 and NS5 regions of the virus polyprotein. HCV core protein and putative matrix proteins have been assayed against human serum samples containing antibodies to HCV and several immunodominant regions within the HCV proteins have been defined. Sallberg, et at., J. Clin. Microbiol., 1992, 30, 1989-1994.

[0005] Protein domains of HCV-1 polyproteins including domains C, E1, E2/NS1, NS2, NS3, NS4, and NS5 have been identified and their approximate boundaries have been provided in WO 93/00365. In addition, individual polypeptides having sequences derived from the structural region of HCV have been designed in order to obtain an immunodominant epitope useful in testing sera of HCV patients. Kotwal, et al., Proc. Natl. Acad. Sci. USA, 1992, 89, 4486-4489.

[0006] The current assay of choice for HCV antibody detection is the Ortho 3.0 ELISA, a manual assay. Chiron-produced recombinant HCV antigens for use in the ELISA are c200 (ns-3, c100), c22 and NS-5. The c33c and c22 antigens are very immunogenic. Antibodies to c33c and c22 are also found in early seroconversion panels. The prevalence of HCV antibodies varies from 58 % to 95% with the highest detection rate obtained for the c33c polypeptide followed by the c22 polypeptide. Chien. et al., Proc. Natl. Acad. Sci. USA, 1992, 89, 10011-10015. However, problems of stabilizing HCVantigens in the liquid phase have been encountered. The lack of stability of HCV antigens in the liquid phase is a major disadvantage of the current HCV antibody detection assay. Therefore, developing an antigen buffer for the anti-HCV immunoassay has been attempted utilizing the same antigens as the Ortho 3.0 ELISA wherein the buffer stabilizes the HCVantigens. In addition, adapting the reagents, buffer and protocols to already existing automated machines, such as the ACS:Centaur has been attempted. Certain aqueous compositions which comprise a biological buffer and a reducing agent are known (WO 96/41164; WO 96/32004; US 5, 616, 460; DE 195 02386; WO 94/25874; JP 06 074956; EP O 341 439).

[0007] Accordingly, there is currently a need to improve the stability of HCV antigens in the liquid phase for use in anti-HCV immunoassays. Such improved assay reagents and methods provide for better detection of HCV antibodies in screening of blood supplies and other biological fluids. It is contemplated that the buffers can be used for other antigens which may be unstable in the liquid phase, e.g. human immunodeficiency virus (HIV) antigens.

SUMMARY OF THE INVENTION

[0008] In one aspect, the present invention is directed to an antigen diluent capable of stabilizing HCV antigens in the liquid phase, in particular HCV recombinant antigens, comprising a reducing agent and a chaotropic agent.

[0009] In another aspect, the present invention is directed to use of an antigen diluent containing a reducing agent, a chaotropic agent and a HCV antigen in an immunoassay for the detection of antibodies directed against Hepatitis C virus.

[0010] In another aspect, an immunoassay kit is provided, the kit comprising a HCV antigen and an antigen diluent containing a reducing agent and a chaotropic agent.

[0011] The invention therefore provides:

- an antigen diluent comprising a Hepatitis C (HCV) antigen, a reducing agent and a chaotropic agent;
- an immunoassay kit for the detection of HCV antibodies wherein the kit comprises a HCV antigen and an antigen diluent comprising a reducing agent and a chaotropic agent; and
- Use of the antigen diluent of the invention in an assay for the detection of antibodies directed against hepatitis C

virus antigens.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The practice of the present invention will employ, unless otherwise indicated, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, et al., Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); DNA Cloning: A Practical Approach, Vols. I & II (D. Glover, ed.); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Handbook of Experimental Immunology, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., Blackwell Scientific Publications); and Fundamental Virology, 2nd Edition, Vols. I & II (B.N. Fields and D.M. Knipe, eds.).

[0013] Reagent stability over time is a critical issue. The c33c antigen diluted in buffer and tested the same day was functional using Magic Lite Assay protocols described below. However the reagent, when stressed at 37°C, lost more than 50% immunoreactivity to early seroconversion panels. The c33c in the liquid phase may slowly "aggregate" or become insoluble. Known components were tried in order to stabilize c33c immunoreactivity such as sugars, gelatin, glycerol, cross-linking reagents and anti-oxidants. It was discovered that keeping the c33c antigen in the reduced form can maintain immunoreactivity for periods over 24 hours, even up to at least 7 days, at 37°C on early c33c seroconversion panels (matching Ortho 3.0 ELISA performance). The reducing agent reduces the disulfide bonds among cysteine groups within the c33c molecule, perhaps improving c33c immunoreactivity and solubility. There was no indication of antigen stability at 37°C C for such lengths of time of conventional lite reagents in the liquid phase prior to the advent of the antigen diluent for c33c. Similar experiments were performed for c200 and a multiple epitope fusion antigen (MEFA-6) as shown below. Thus, the present invention provides antigen diluents or buffers for stabilizing HCV antigens for use in anti-HCV immunoassays. The antigen diluents or buffers of the present invention can be used in immunoassays such as, for example, ELISA and CLIA.

[0014] The present invention is directed to antigen diluents or buffers providing for improved stability of HCV antigens in the liquid phase. As used herein, "antigen diluents or buffers" refers to the solution in which the antigen is contained; it may or may not possess buffering capacity. In particular, the invention is directed to antigen diluents or buffers for improved stability for the recombinant HCV antigens in the Ortho 3.0 ELISA, and the like. The present invention was achieved by adding a reducing agent such as, for example, dithiothreitol (DTT) to the antigen diluent or buffer.

[0015] In a preferred embodiment of the invention, the HCV antigen diluent or buffer comprises a reducing agent. In another preferred embodiment of the invention, the HCV antigen diluent or buffer comprises sodium phosphate (pH 6.5), ethylenediaminetetraacetic acid (EDTA), DTT, gelatin, ammonium thiocyanate, sodium azide and SDS. However, these individual reagents can be replaced by similar reagents performing essentially the same function. For example, DTT can be replaced with additional reducing agents such as, for example, thioglycerol, mercaptoethenol, and the like. Sodium phosphate can be replaced by sodium borate and other buffers. Gelatin can be replaced with BSA and other blocking agents of non-specific binding. Sodium thiocyanate can be replaced with ammonium thiocyanate and other chaotropic agents. SDS can be replaced by a number of detergents such as, for example, Tween-20, and other detergents. Sodium azide can be replaced by other anti-bacterial agents. In addition, EDTA can be replaced by ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) and other chelating agents. One skilled in the art is familiar with reagents which can be substituted for those of the present invention.

[0016] In a preferred embodiment of the present invention, the HCV antigen diluent comprises from about 15 mM to about 100 mM sodium phosphate, pH 6.5. More preferably the diluent comprises from about 20 mM to about 75 mM sodium phosphate, pH 6.5. Most preferably, the diluent comprises 24 or 25 mM sodium phosphate, pH 6.5.

[0017] In another preferred embodiment of the present invention, the HCV antigen diluent comprises from about 1 mM to about 10 mM EDTA. More preferably the diluent comprises from about 3 mM to about 7 mM EDTA. Most preferably, the diluent comprises 5 mM EDTA.

[0018] In another preferred embodiment of the present invention, the HCV antigen diluent comprises from about 1 mM to about 200 mM DTT. More preferably the diluent comprises from about 5 mM to about 100 mM DTT. Most preferably, the diluent comprises 10 mM DTT.

[0019] In another preferred embodiment of the present invention, the HCV antigen diluent comprises from about 0.05 % to about 1 % gelatin. More preferably the diluent comprises from about 0.1% to about 0.5 % gelatin. Most preferably, the diluent comprises 0.2 % gelatin.

[0020] In another preferred embodiment of the present invention, the HCV antigen diluent comprises from about 10 mM to about 500 mM ammonium thiocyanate. More preferably the diluent comprises from about 50 mM to about 200 mM ammonium thiocyanate. Most preferably, the diluent comprises 100 mM ammonium thiocyanate.

[0021] In another preferred embodiment of the present invention, the HCV antigen diluent comprises from about 0.01% to about 0.3% sodium azide. More preferably the diluent comprises from about 0.05% to about 0.2% sodium azide. Most preferably, the diluent comprises 0.09% sodium azide.

[0022] In another preferred embodiment of the present invention, the HCV antigen diluent comprises from about 0.01 % to about 0.5 % SDS. More preferably the diluent comprises from about 0.05% to about 0.2 % SDS. Most preferably, the diluent comprises 0.1 % SDS.

[0023] In another preferred embodiment of the present invention, the HCV antigen diluent for the manual assay comprises 25 mM sodium phosphate, pH 6.5, 5 mM EDTA, 10 mM DTT, 0.2% gelatin, 100 mM ammonium thiocyanate. 0.09% sodium azide and 0.1% SDS.

[0024] For the automated assays, a preferred antigen buffer for c33c comprises 50 mM phosphate, 5 mM EDTA, 100 mM ammonium thiocyanate, 0.06% SDS, 0.25% fish gelatin and 10 mM DTT.

[0025] Table 1 shows a preferred HCV buffer.

Table 1: HCV Antigen Buffer For HCV Antigens

Description	Concentration	Source	Product#	Lot#
Sodium Phosphate Monobasic	25 mM	JT Baker	3818-05	A45101
Sodium Azide	0.09%	Fisher Biotech	BP922-500	953331
EDTA	5 mM	Fisher Chemical	S311-100	953493
Sodium Thiocyanate	100 mM	Sigma	S-7757	96HO543
Tween-20	0.10%	Sigma	P-1379	56HO876
Gelatin (fish)	0.20%	Sigma	G-7765	45H1157
DTT	10 mM	Sigma	D-5545	26HO3801

[0026] The HCV antigen diluents or buffers of the present invention can be prepared by well known media preparation techniques. A preferred embodiment of preparing the HCV antigen diluents of the present invention is shown in Table 2.

Table 2: Process For Preparation Of Diluents

Process Step	Amount
1. Add 95 % of batch quantity P-30 water	
2. Add sodium phosphate, monobasic	3.45 g/L
3. Add sodium azide	0.9 g/L
4. Add EDTA	1.86 g/L
5. Add sodium thiocyanate	8.1 g/L
6. pH solution 6.5 ± 0.1 and stir	
7. Add Tween-20	1 mL/L
8. Add gelatin	2 mL/L
9. Add DTT	1.54 g/L
10. Stir solution until dissolved	
11. Filter through 1.22 µm Millipak filter unit	
12. Store at 4°C in dark	

[0027] The HCV antigen diluents or buffers of the present invention can be used in manual or automatic assays. The antigen diluents or buffers of the present invention can be used with numerous HCV antigens including, but not limited to, c33c, MEFA-6, c22p, c100p, NS-5 and c200. These HCV antigens can be prepared by recombinant procedures routinely used in the art.

[0028] HCV c33c (NS3) and c100 (NS4) region sequences contain epitopes from the immunodominant core and were prepared as described in Chien, et al., Proc. Natl. Acad. Sci. USA, 1992, 89, 10011-10015. The c200 antigen is a fusion protein consisting of the c33c and c100 antigens. The c22 (119 amino acids) and NS5 (942 amino acids) antigens were expressed as internal antigens within the yeast *S. cerevisiae* as C-terminal fusions with human superoxide dismutase (SOD) using methods described previously for the generation of the c100-3 (363 amino acids) antigen. Kuo, et al.,

Science, 1989, 244, 362-364; and Cousens, et al., Gene, 1987, 61, 265-275. The c33c antigen (363 amino acids) was expressed as an internal SOD fusion polypeptide in *E. coli* by methods described for the synthesis of 5-1-1 antigen. Choo, et al., Science, 1989, 244, 359-362. The recombinant HCV antigens were purified as described in Chien, et al., Proc. Natl. Acad. Sci. USA, 1989, 89, 10011-10015. In the present invention, all HCV antigens were prepared as SOD fusion proteins. However, other suitable fusion proteins can be made depending upon the availability of appropriate antibodies that recognize the fusion partner.

[0029] MEFA-6 contains epitopes from the core, envelope, NS3, NS4 and NS5 regions of the hepatitis C polyprotein, including equivalent antigenic determinants from HCV strains 1, 2, and 3. The various DNA segments coding for the HCV epitopes were constructed by PCR amplification or by synthetic oligonucleotides. Table 3, below, describes the amino acid segments of each epitope, the linear arrangement of the various epitopes and the number of copies in the MEFA-6 cassette. MEFA-6 cassette was prepared as described in application PCT US97/08950 filed May 23, 1997.

[0030] As shown in Table 3, the MEFA-6 antigen includes multiple copies of HCV epitopes from the core and NS5 region; different serotype epitopes from the NS4 5-1-1 region; a single copy of major linear epitopes from the c100 C-terminal regions, E1, and E2 regions, as well as the HCV NS3 (c33c) region. The general structural formula for MEFA-6 is hSOD-E1-E2-c33c-5-1-1(type 1)-5-1-1(type 3)-5-1-1(type 2)-c100-NS5(2 copies)-core (2 copies). This antigen has a very high expression level in yeast, purifies to a high degree of homogeneity, and exhibits high sensitivity and high selectivity in the immunoassays described below. MEFA-6 was prepared as described in application Serial No. 08/859,524 filed May 20, 1997.

Table3: MEFA-6 Antigen Epitopes And Their Location Within The HCV Genome

MEFA aa#	5' End Site	Epitope	HCV aa#	Strain
1-154	*Nco*I	hSOD		
159-176	*Eco*RI	E1	303-320	1
179-217	*Hin*dIII	E2	405-444	1
218-484	*Dra*III	c33c	1192-1457	1
487-533	*Sph*I	5-1-1	1689-1735	1
536-582	*Nru*I	5-1-1	1689-1735	3
585-631	*Cla*I	5-1-1	1689-1735	2
634-673	*Ava*I	c100	1901-1940	1
676-711	*Xba*I	NS5	2278-2313	1
714-749	*Bgl*II	NS5	2278-2313	1
750-793	*Nco*I	core	10-53	1
796-839	*Sac*I	core	10-53	1

[0031] The detectable marker may include, but is not limited to, a chromophore, an antibody, an antigen, an enzyme, an enzyme reactive compound whose cleavage product is detectable, rhodamine or rhodamine derivative, biotin, streptavidin, a fluorescent compound, a chemiluminscent compound, derivatives and/or combinations of these markers. In the present examples, the chemiluminescent compound dimethyl acridinium ester (DMAE, Ciba Corning Diagnostics Corp.) was used. Labeling with any marker is carried out under conditions for obtaining optimal detection and antigenicity of the MEFA-6 or other epitope. Where DMAE is the detectable marker in an assay, the resultant HCV r-Ag-DMAE conjugate is the tracer, with DMAE detectable by light emission when reacted with $NaOH/H_2O_2$.

[0032] A polypeptide, antibody or synthetic peptide antigen was labeled with DMAE by reaction of amino acid side chains (e.g. lysine e side chain or cysteine thiol) with a reactive moiety covalently linked to DMAE (see WO 95/27702, published October 19, 1995, Ciba Corning Diagnostics Corp.). For example, the HCV antigens described herein were labeled by reaction with the amino groups of lysine side chains with NSP-DMAE-NHS (2',6'-Dimethyl-4'-(N-succinimidyloxycarbonyl)phenyl-10-(3'-Sulfopropyl)-acridinium-9-carboxylate) obtained from Ciba Corning. Thiols of amino acid side chains can be labeled using DMAE-ED-MCC or NSP-DMAE-PEG-BrAc (Ciba Corning). Labeling procedures were generally as described in WO 95/27702 with variations in conditions as necessary for each antigen to provide optimal detection and antigenicity.

EXAMPLES

Example 1: Manual Assay

[0033] A Magic Lite Analyzer System II (MLA II) is used for the manual assay. Parameters such as volume, concentration, time, and temperature are provided for guidance, but may be adjusted accordingly. Briefly, a 10 µl aliquot of test sample was added to corresponding tubes. The test sample is preferably a biological fluid (plasma or serum, for example) possibly containing anti-HCV antibodies, as well as proper controls. To each tube is added 100 µl of antigen diluent or buffer and incubated for 6 minutes at 37°C. To each tube is added 100 µl of solid phase buffer containing paramagnetic particles (PMP) conjugated to rat anti-human IgG antibodies (PMP/anti-human IgG) for a final concentration of approximately 60 µg/assay. However, other anti-human IgG antibodies are suitable. Preferably, the paramagnetic particles are less than approximately 10 µm in diameter. The PMP/anti-human IgG particles can be diluted in a diluent containing Tris buffer, pH 8.0, 150 mM NaCl, 2.75 % BSA, 0.1 % casein, 0.1 % Tween-20, 0.1% yeast extract, 0.25% *E. coli* extract, 0.005% SOD, 0.09% NaN_3 and 1 mM EDTA. Subsequently, recombinant HCV antigens (HCV antigen/SOD fusion proteins) conjugated to DMAE (MEFA-6-DMAE, c33c-DMAE and c200-DMAE, for example) are added in a 50 µl volume of ligand reagent (LR) diluent at a concentration of approximately 0.1 µg/assay to 1 µg/assay. Preferably, an amount of ligand reagent is added to each sample such that approximately 25 x 10^6 light unit equivalents (relative light units, RLU) are present per assay. This approximate amount of light unit equivalents is preferred for the addition of a single ligand, or for multiple ligands. LR diluent contains Tris buffer, pH 8.0, 150 mM NaCl, 1.0 % BSA, 0.1 % Tween-20, 0.09% NaN_3, and 1 mM EDTA. To ensure complete mixing, the tubes are shaken on a Vortex mixer 6 times at 5-10 seconds each time. The sample tubes are incubated at 37°C for 18 minutes. The sample tubes are placed on a magnet for 3 minutes, for sufficient time to sediment the PMP particles. The samples are decanted using a magnet to retain the PMP particles. The PMP particles are washed twice with vortexing in 1 ml of PBS. The wash solution is PBS, 0.1% Tween-20, 0.09% NaN_3, and 1 mM EDTA. The steps of mixing, incubating, sedimenting and decanting may be repeated at least one time. To each tube 100 µl of water is added to resuspend the PMP particles. The tubes are then placed in an MLA-II instrument and light emission is measured for 2 seconds.

Example 2: Automated Assay

[0034] The manual anti-HCV assay described above was adapted for automated use using an ACS:Centaur apparatus. The following procedure is used. Briefly, the ACS:Centaur system automatically performs the following steps: 1) dispenses 10 µl of sample into a cuvette; 2) dispenses 100 µl of ancillary diluent buffer, 100 µl of Lite Reagent/Solid Phase, 50 µl of antigen reagent 2 (e.g., MEFA-6), 50 µl of antigen reagent 1 (e.g., c33c) and incubates the mixture for 18 minutes at 37°C; 3) separates the solid phase from the mixture and aspirates the unbound reagent; 4) washes the cuvette with wash reagent 1; 5) dispenses 300 µl each of acid reagent and base reagent to initiate the chemiluminescent reaction; and 6) reports results according to the selected option, as described in the system operating instructions or in the online help system. The solid phase/Lite reagent diluent buffer comprises 50 mM Tris, 0.5 M KCl, 1 mM EDTA, 3.75 % BSA, 0.003% Yeast, 0.05 g/L *E. coli,* 0.5 % Tween-20, 2 mg/L Amphotericin B, 24 mg/L Gentamicin Sulfate, 30 µg/test Solid Phase and 45 x 10^6 test Lite Reagent(anti-SOD*DMAE antibodies). The ancillary diluent buffer comprises 50 mM Tris, 0.5M KCl, 1 mM EDTA, 3.75% BSA, 0.003% Yeast, 0.05 g/L *E. coli,* 0.5% Tween-20, 2 mg/L Amphotericin B, 24 mg/L Gentamicin Sulfate, 0.05 g/L Ascites IgG1 and 0.1 g/L Ascites 1gG2A (blocking antibodies). The wash reagent comprises PBS/Tween-20. The acid reagent comprises 0.5% H_2O_2/0.1 N HNO_3. The base reagent comprises <0.25N NaOH with surfactant.

Example 3: Manual Assay With c33c

[0035] A manual assay using c33c HCV antigen was performed with 100 ng of c33c per assay using the methodology described above in Example 1. The antigen diluent comprised 25 mM sodium phosphate, pH 6.5, 100 mM sodium thiocyanate, 5 mM EDTA, 0.1% Tween-20, 0.2 % fish gelatin, 0.09% sodium azide and 10 mM DTT. The assay was performed with 3 x 10^6 RLU/10 µl, 30 µg/assay PMP. The assay was performed at varying times and under varying temperatures. For example, the assay was performed at Day 0 at 4°C, at Day 3 at 4°C, at Day I at 37°C, at Day 2 at 37°C, at Day 3 at 37°C and at Day 6 at 37°C.

[0036] A 10 µl sample (such as a biological fluid containing human anti-HCV antibodies) was added to each sample tube. Samples included: random negative controls (r1, r2 and r3), a positive control (Virotrol), seroconversion panels (PHV905-5, PHV907-4 and PHV904-6), HCV patient samples (FF25931) and seroconversion samples (6214-09 and 6212-04). The results are shown in Table 4. Sensitivity was reported as the optical density of the assay sample divided by the assay detection cut off in optical density units (s/co). All known negative samples exhibited relative light units (RLU) below the cutoff value, while known positive samples exhibited RLUs well above the cutoff value.

[0037] For comparative purposes, the detection of HCV antibodies from some of the samples (see Table 4) was also performed by Ortho 3.0 and a commercial strip immunoblot assay (RIBA* 3.0 Chiron Corporation), which assay is used clinically as a confirmatory test for HCV antibody detection. According to the RIBA* method, recombinant HCV antigens are separated by gel electrophoresis and contacted with patient serum. Reactivity with the separated antigens is performed by immunoblot assay using secondary labeled antibodies. Assay results are scored on a plus/minus scale. Eheling, et al., Lancet, 1991, 337, 912-913. The Ortho 3.0 assay was performed according to the manufacturer's instructions. c33c, c22p, c100p, and NS-5 were used as the HCV antigens for these tests.

[0038] Briefly, the RIBA* 3.0 assay was performed as follows. Approximately 30 minutes before beginning the assay, the kit was removed from refrigeration (2 to 8°C) and the components of the kit allowed to come to room temperature (15 to 30°C). The required number of strips were removed from the sealed foil pouches and placed in the assay tube rack in their respective tubes. One ml of Specimen Diluent was added to each tube so that the entire strip was covered with liquid. Twenty µl of the appropriate specimen or control was added to the corresponding tube. The tubes were capped and inverted to mix. The rack with the tubes was placed on a rocker and fastened with rubber bands or tape; the rack was rocked (at 16-20 cycles/minute) for 4 to 4½ hours at room temperature (15 to 30°C). The tubes were uncapped and the liquid was completely aspirated into a waste container. One ml of Specimen Diluent was added to each tube. The tubes were capped and placed on the rack on the rocker and rocked for 30 to 35 minutes at room temperature. The liquid was then aspirated. One ml of Working Wash Buffer was added to each tube, then the liquid and strips poured into wash vessels containing 30 ml of Working Wash Buffer (maximum 20 strips per wash vessel). The wash vessels were completely filled with Working Wash Buffer (60 mL total volume), then the wash was decanted. To retain the strips, the wash vessel was gently rolled while decanting. Sixty ml of Working Wash Buffer was added, swirled, then the wash was decanted the while retaining the strips. One ml of Conjugate per strip was added to each wash vessel (minimum 10 ml per wash vessel). The wash vessels were rotated on a rotary shaker at 110 ± 5 rpm for 9 to 11 minutes at room temperature (15 to 30°C). Working Substrate was prepared up to 1 hour prior to use. Upon completion of Conjugate incubation, the Conjugate was decanted and the strips were washed by adding 60 ml of Working Wash Buffer and swirling. The wash was decanted and this step was repeated two more times. The final wash was decanted. One ml of Working Substrate was added per strip to each wash vessel (minimum 10 ml per wash vessel). The wash vessels were rotated on a rotary shaker at 110 ± 5 rpm for 15 to 20 minutes at room temperature (15 to 30°C). The Working Substrate was decanted and the strips were washed by adding 60 ml of distilled or deionized water and swirling. The wash was decanted and this step was repeated one more time. To retain strips, the wash vessel was gently rolled while decanting. Using forceps, the strips were transferred to absorbent paper and excess water was blotted. The strips were air-dried in the dark for at least 30 minutes at room temperature. The strips were interpreted within 3 hours. Anti-HCV reactivity in a specimen was determined by comparing the intensity of each antigen band to the intensity of the human IgG (Level I and Level II) internal control bands on each strip. The identity of the antibodies was defined by the specified location of the antigen band. The intensity of the antigen/peptide bands was scored in relation to the intensities of the internal IgG controls as follows: absent (-), less than intensity of the Level I IgG control band (-/+), equal to intensity of the Level I IgG control band (1+), greater than intensity of the Level I IgG control band and less than intensity of the Level II IgG control band (2+), equal to intensity of the Level II IgG control band (3+), and greater than intensity of the Level II IgG control band (4+).

Example 4: Manual Assay With c200

[0039] A manual assay using c200 HCV antigen was performed as described in Example 1 with various amounts of reducing agent. The stabilizing buffer was the same as in Example 3, except for the amount of reducing agent. The assay was performed with 3 x 10^6 RLU/10 µl, 30 µg/assay PMP. The assay was performed at varying times and under varying amounts of reducing agent. For example, the assay was performed after 1 day at 37°C with 20 mM DTT (Vial I), after 1 day at 37°C without DTT (Vial II), and after 1 day at 37°C where 20 mM DTT was added prior to testing (Vial III). Vials II and III were also tested after 3 days.

[0040] A 10 µl sample (such as a biological fluid containing human anti-HCV antibodies) was added to each sample tube. Samples included: random negative controls (r1, r2, r3, r4 and r5), seroconversion panels (PHV904-6 and PHV906-1) and HCV patient samples (FF25931) at various dilutions. The results are shown in Table 5. s/n is the sensitivity divided by the value ave.neg.

Example 5: Manual Assay With MEFA-6 And c33c

[0041] A manual assay using MEFA-6 and c33c HCV antigen was performed with 100 ng of MEFA-6 and 85 ng of c33c per assay using the methodology described above in Example 1. The stabilizing buffer for MEFA-6 comprised 50 mM sodium borate, pH 9.5, 5 mM EDTA, 0.05% Tween-20, 0.5% BSA, and 1 % thioglycerol. At this pH 9.5 MEFA-6 is stable so no reducing agent is necessary. The buffer for c33c comprised 25 mM sodium phosphate, pH 6.5, 5 mM EDTA,

0.1% Tween-20, 0.2% fish gelatin, 100 mM sodium thiocyanate, and 10 mM DTT. The assay was performed with 4.5 x 10^6 RLU/10 µl of anti-SOD*DMAE, 30 µg/assay PMP. The assay was performed at varying times and under varying temperatures. For example, the assay was performed at Day 7 at 4°C and at Day 7 at 37°C.

[0042] A 10 µl sample (such as a biological fluid containing human anti-HCV antibodies) was added to each sample tube. Samples included, random negative controls (r1, r2, r3 and r4), a positive control (Virotrol), seroconversion panels (PHV905-5, PHV909-1, PHV909-2 and PHV909-3), seroconversion samples (6212-02 and 6214-09) and seroconversion control panels (SC-0030A, SC-0030B, SC-0030C, SC-0030D, SC-0040A, SC-0040B, SC-0040C, SC-0040D and SC-0040E). The results are shown in Table 6. Sensitivity was reported as the optical density of the assay sample divided by the assay detection cut off in optical density units (s/co). All known negative samples exhibited relative light units (RLU) below the cutoff value, while known positive samples exhibited RLUs well above the cutoff value.

[0043] For comparative purposes, the detection of HCV antibodies from some of the samples (see Table 6) was also performed by Ortho 3.0 and RIBA* 3.0 as described in Example 3.

Example 6: Manual Assay With MEFA-6

[0044] A manual assay using MEFA-6 HCV antigen was performed with 100 ng of MEFA-6 per assay using the methodology described above in Example 1. The buffer for MEFA-6 comprised 50 mM sodium borate, pH 9.5, 5 mM EDTA, 0.05% Tween-20, 0.5 % BSA, and 1% thioglycerol. The assay was performed with 4.5 x 10^6 RLU/10 µl of anti-SOD*DMAE, 30 µg/assay PMP. The assay was performed at Day 7 at 4°C.

[0045] A 10 µl sample (such as a biological fluid containing human anti-HCV antibodies) was added to each sample tube. Samples included, random negative controls (r1, r2 and r3), positive control (Virotrol) and seroconversion control panels (SC-0030A, SC-0030B, SC-0030C and SC-0030D). The results are shown in Table 7. Sensitivity was reported as the optical density of the assay sample divided by the assay detection cut off in optical density units (s/co). All known negative samples exhibited relative light units (RLU) below the cutoff value, while known positive samples exhibited RLUs well above the cutoff value.

[0046] For comparative purposes, the detection of HCV antibodies from some of the samples (see Table 7) was also performed by Ortho 3.0 and RIBA* 3.0 as described above. The foregoing examples are meant to illustrate the invention and are not to be construed to limit the invention in any way.

Table 4: c33c Assay

Sample	Day 0 4°C	Day 3 4°C	Day 1 37°C	Day 2 37°C	Day 3 37°C	Day 6 37°C
r1	1925	1247	1001	1509	1971	2202
r2	1740	1679	1632	1448	1401	1863
r3	1602	1432	1463	1401	1725	1940
Virotrol	61708	64156	65604	60060	56595	59044
6214-09	14322	16555	10888	16555	14784	15246
6212-04	40856	43351	41842	40225	36421	39008
PHV905-5	10734	15030	14969	13721	15785	13999
PHV907-4	2341	1756	1940	2017	2110	2002
PHV904-6	53299	49496	54208	54285	47155	45676
FF25931 1:8	567120	608993	530006	572603	568974	581504
ave.neg.	1756	1453	1365	1453	1699	2002
cutoff	5267	4358	4096	4358	5097	6005

											ORTHO
	s/co	s/co	s/co	s/co	s/co	s/co	RIBA	3.0			3.0
Virotrol	11.72	14.72	16.02	13.78	11.10	9.83	c33c	c22p	c100p	NS-5	s/co
6214-09	2.72	3.80	2.66	3.80	2.90	2.54	2 +	-	+/-	-	0.9
6212-04	7.76	9.95	10.22	9.23	7.15	6.50	1 +	-	-	-	1.4
PHV905-5	2.04	3.45	3.65	3.15	3.1	2.33	1 +	-	-	-	0.9
PHV907-4	0.44	0.40	0.47	0.46	0.41	0.33	-	1+	-	-	0.1
PHV904-6	10.12	11.36	13.23	12.46	9.25	7.61	2+	-	-	-	>5.0

Table 5: c200 Assay

Sample	Vial I 37°C Day control c200 20mM DTT	Vial II 1 37°C Day test c200 w/o DTT	Vial III 1 37°C Day 1 test c200 added 20mM DTT	Vial III 37°C Day 1 test c200 added 40mM DTT	Vial III 37°C Day 3 test c200 added 40mM DTT	Vial II 37°C Day 3 test c200 w/o DTT
random r1	1463	1448	1078	1109	1217	801
random r2	1217	1324	1247	1879	1309	1016
random r3	1155	1247	1340	1217	1063	1124
random r4	1170	1217	2402	1340	1386	1140
random r5	1155	1232	1217	1494		
serocon. PHV904-6	29106	10102	8763	13182	14060	2141
serocon. PHV906-1	27828	15231	16016	21468	30523	25656
FF25931 1:4	643551	574944	435543	318025	322307	277616
FF25931 1: 256	26657	19774	16339	16524	22484	21699
FF25931 1: 1024	9948	7854	8516	8408	12859	17048
ave.neg.	1232	1294	1457	1408	1244	1020
	s/n	s/n	s/n	s/n	s/n	s/n
serocon. PHV904-6	23.6	7.8	6.0	9.4	11.3	2.1
serocon. PHV906-1	22.6	11.8	11.0	15.2	24.5	25.1
FF25931 1:4	522.4	444.5	299.0	225.9	259.1	272.1
FF25931 1: 256	21.6	15.3	11.2	11.7	18.1	21.3
FF25931 1: 1024	8.1	6.1	5.8	6.0	10.3	16.7

Table 6: MEFA-6 + c33c Assay

Sample	4°C	Day 7	37°C	Day 7	Ortho 3.0	RIBA	3.0			
	s	s/co	s	s/co	s/co	c100p	c33c	c22p	NS-5	Genotype
NABI SC-0030A	6607	0.39	5960	0.43	0.005	-	-	-	-	1a
NABI SC-0030B	14522	0.86	8778	0.64	0.015	3+	+/-	+/-	-	
NABI SC-0030C	86748	5.12	46785	3.40	1.837	4+	1 +	2+	-	
NABI SC-0030D	472749	27.92	489304	35.54	4.900	4+	4+	4+	3+	
NABI SC-0040A	9379	0.55	7454	0.54	0.003	-	-	-	-	2b

(continued)

Sample	4°C	Day 7	37°C	Day 7	Ortho 3.0	RIBA	3.0			
	s	s/co	s	s/co	s/co	c100p	c33c	c22p	NS-5	Genotype
NABI SC-0040B	12720	0.75	7546	0.55	0.056	-	-	-	-	
NABI SC-0040C	65927	3.89	29799	2.16	1.215	+/-	2+	-	-	
NABI SC-0040D	106845	6.31	43613	3.17	1.534	+/-	2+	-	-	
NABI SC-0040E	175067	10.34	78124	5.67	3.247	1 +	3+	1 +	-	
random r1	5236		4697							
random r2	5652		4112							
random r3	5991		5375							
random r4	5698		4173		Ortho 3.0	RIBA	3.0			
Sample	4°C	Day	7 37°C	Day 7	Ortho 3.0	RIBA	3.0			
	s	s/co	s	s/co	s/co	c100p	c33c	c22p	NS-5	Genotype
control Virotrol 1	117548	6.94	74721	5.43	s/co	c100p	c33c	c22p	NS-5	
BCP 6212-04	68807	4.06	31616	2.30	1.4	-	1 +	-	-	
BCP 6214-09	81543	4.82	24270	1.76	0.9	+/-	2+	-	-	
BBI PHV905-5	25040	1.48	16755	1.22	0.9	-	1 +	-	-	
BBI PHV909-1	6699	0.40	5313	0.39	0.0	-	-	-	-	
BBI PHV909-2	30661	1.81	15646	1.14	1.3	-	-	1 +	+/-	
BBI PHV909-3	32432	1.92	16570	1.20	1.4	-	-	2+	+/-	
ave.neg.	5644		4589							
cutoff	16933		13768							

Table 7: MEFA-6 Assay

Sample	4°C	Day 7						
	s	s/co						
random r1	8624							
random r2	8609							
random r3	7192		Ortho 3.0	RIBA 3.0				
control Virotrol 1	129406	5.00	s/co	c100p	c33c	c22p	NS-5	Genotype
NABI SC-0030A	8516	0.33	0.005	-	-	-	-	1a
NABI SC-0030B	26827	1.04	0.015	3+	+/-	+/-	-	

(continued)

Sample	4°C	Day 7						
	s	s/co						
NABI SC-0030C	179980	6.96	1.837	4+	1 +	2+	-	
NABI SC-0030D	508831	19.67	4.900	4+	4+	4+	3+	
ave.neg.	8624							
cutoff	25872							

Claims

1. An antigen diluent comprising a Hepatitis C (HCV) antigen, a reducing agent and a chaotropic agent.
2. The antigen diluent of claim 1 wherein the reducing agent is selected from dithiothreitol, thioglycerol and mercaptoethanol.
3. The antigen diluent of claim 1 wherein the reducing agent is dithiothreitol (DTT).
4. The antigen diluent of claim 3 wherein the concentration of DTT is from 1mM to 200mM.
5. The antigen diluent of claim 3 wherein the concentration of DTT is from 5mM to 100mM.
6. The antigen diluent of claim 3 wherein the concentration of DTT is about 10mM.
7. The antigen diluent of claim 1 further comprising a buffering agent.
8. The antigen diluent of claim 7 wherein the buffering agent is selected from sodium phosphate or sodium borate.
9. The antigen diluent of claim 8 wherein the buffering agent is sodium phosphate.
10. The antigen diluent of claim 9 wherein the concentration of sodium phosphate, pH 6.5, is from 15mM to 100mM.
11. The antigen diluent of claim 1 further comprising a detergent.
12. The antigen diluent of claim 11 wherein the detergent is selected from sodium dodecyl sulfate (SDS) and Tween-20®.
13. The antigen diluent of claim 12 wherein the detergent is SDS.
14. The antigen diluent of claim 13 wherein the concentration of SDS is from 0.01% to 0.5%.
15. The antigen diluent of claim 1 further comprising an anti-bacterial agent.
16. The antigen diluent of claim 15 wherein the anti-bacterial agent is sodium azide.
17. The antigen diluent of claim 16 wherein the concentration of sodium azide is from 0.01% to 0.3%.
18. The antigen diluent of claim 1 further comprising a chelating agent.
19. The antigen diluent of claim 18 wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).
20. The antigen diluent of claim 19 wherein the concentration of EDTA is from 1mM to 10mM.
21. The antigen diluent of claim 1 further comprising a blocking agent of non-specific binding.
22. The antigen diluent of claim 21 wherein the blocking agent of non-specific binding is selected from gelatin and bovine

serum albumin.

23. The antigen diluent of claim 22 wherein the blocking agent of non-specific binding is gelatin.

24. The antigen diluent of claim 23 wherein the concentration of gelatin is from 0.05% to 1.0%.

25. The antigen diluent of claim 1 wherein the chaotropic agent is selected from sodium thiocyanate and ammonium thiocyanate.

26. The antigen diluent of claim 25 wherein the chaotropic agent is ammonium thiocyanate.

27. The antigen diluent of claim 26 wherein the concentration of ammonium thiocyanate is from 10mM to 500mM.

28. The antigen diluent of claim 1 further comprising a buffering agent, a chelating agent, a blocking agent of non-specific binding , an antibacterial agent, and a detergent.

29. The antigen diluent of claim 28 wherein said buffering agent is sodium phosphate, said chelating agent is EDTA, said blocking agent of non-specific binding is gelatin, said chaotropic agent is sodium thiocyanate, said antibacterial agent is sodium azide, and said detergent is SDS.

30. The antigen diluent of claim 29 comprising 25mM sodium phosphate, pH 6.5, 5mM EDTA, 10mM DTT, 0.2% gelatin, 100mM ammonium thiocyanate, 0.09% sodium azide and 0.1% SDS.

31. The antigen diluent of claim 29 comprising 50mM sodium phosphate, 5mM EDTA, 100mM ammonium thiocyanate, 0.06% SDS, 0.25% fish gelatin and 10mM DTT.

32. The antigen diluent of claim 1 wherein said Hepatitis C (HCV) antigen is c33c, MEFA-6, c22p, c100p, NS-5 or c200.

33. An immunoassay kit for the detection of HCV antibodies wherein the kit comprises a HCV antigen and an antigen diluent comprising a reducing agent and a chaotropic agent.

34. The immunoassay kit of claim 33 wherein the reducing agent is selected from dithiothreitol, thioglycerol and mercaptoethanoL

35. The immunoassay kit of claim 34 wherein the reducing agent is dithiothreitol.

36. Use of the antigen diluent of claim 1 in an assay for the detection of antibodies directed against Hepatitis C virus antigens.

Patentansprüche

1. Antigen-Verdünnungsmittel, umfassend ein Hepatitis C (HCV)-Antigen, ein Reduktionsmittel und ein chaotropes Mittel.

2. Antigen-Verdünnungsmittel gemäß Anspruch 1, wobei das Verdünnungsmittel aus Dithiothreitol, Thioglycerin und Mercaptoethanol ausgewählt ist.

3. Antigen-Verdünnungsmittel gemäß Anspruch 1, wobei das Reduktionsmittel Dithiothreitol (DTT) ist.

4. Antigen-Verdünnungsmittel gemäß Anspruch 3, wobei die Konzentration an DTT 1 mM bis 200 mM ist.

5. Antigen-Verdünnungsmittel gemäß Anspruch 3, wobei die Konzentration an DTT 5 mM bis 100 mM ist.

6. Antigen-Verdünnungsmittel gemäß Anspruch 3, wobei die Konzentration an DTT etwa 10 mM ist.

7. Antigen-Verdünnungsmittel gemäß Anspruch 1, das außerdem ein Puffermittel umfasst.

8. Antigen-Verdünnungsmittel gemäß Anspruch 7, wobei das Puffermittel aus Natriumphosphat und Natriumborat ausgewählt ist.

9. Antigen-Verdünnungsmittel gemäß Anspruch 8, wobei das Puffermittel Natriumphosphat ist.

10. Antigen-Verdünnungsmittel gemäß Anspruch 9, wobei die Konzentration von Natriumphosphat, pH 6,5, 15 mM bis 100 mM ist.

11. Antigen-Verdünnungsmittel gemäß Anspruch 1, das außerdem ein Detergens umfasst.

12. Antigen-Verdünnungsmittel gemäß Anspruch 11, wobei das Detergens aus Natriumdodecylsulfat (SDS) und Tween-20® ausgewählt ist.

13. Antigen-Verdünnungsmittel gemäß Anspruch 12, wobei das Detergens SDS ist.

14. Antigen-Verdünnungsmittel gemäß Anspruch 13, wobei die Konzentration an SDS 0,01% bis 0,5% ist.

15. Antigen-Verdünnungsmittel gemäß Anspruch 1, das außerdem ein antibakterielles Mittel umfasst.

16. Antigen-Verdünnungsmittel gemäß Anspruch 15, wobei das antibakterielle Mittel Natriumazid ist.

17. Antigen-Verdünnungsmittel gemäß Anspruch 16, wobei die Konzentration von Natriumazid 0,01% bis 0,3% ist.

18. Antigen-Verdünnungsmittel gemäß Anspruch 1, das außerdem einen Chelatbildner umfasst.

19. Antigen-Verdünnungsmittel gemäß Anspruch 18, wobei der Chelatbildner Ethylendiamintetraessigsäure (EDTA) ist.

20. Antigen-Verdünnungsmittel gemäß Anspruch 19, wobei die Konzentration von EDTA 1 mM bis 10 mM ist.

21. Antigen-Verdünnungsmittel gemäß Anspruch 1, das außerdem ein Blockierungsmittel für nichtspezifische Bindung umfasst.

22. Antigen-Verdünnungsmittel gemäß Anspruch 21, wobei das Blockierungsmittel für nichtspezifische Bindung aus Gelatine und Rinderserumalbumin ausgewählt ist.

23. Antigen-Verdünnungsmittel gemäß Anspruch 22, wobei das Blockierungsmittel für nichtspezifische Bindung Gelatine ist.

24. Antigen-Verdünnungsmittel gemäß Anspruch 23, wobei die Konzentration von Gelatine 0,05% bis 1,0% ist.

25. Antigen-Verdünnungsmittel gemäß Anspruch 1, wobei das chaotrope Mittel aus Natriumthiocyanat und Ammoniumthiocyanat ausgewählt ist.

26. Antigen-Verdünnungsmittel gemäß Anspruch 25, wobei das chaotrope Mittel Ammoniumthiocyanat ist.

27. Antigen-Verdünnungsmittel gemäß Anspruch 26, wobei die Konzentration von Ammoniumthiocyanat 10 mM bis 500 mM ist.

28. Antigen-Verdünnungsmittel gemäß Anspruch 1, das außerdem ein Puffermittel, einen Chelatbildner, ein Blockierungsmittel für nichtspezifische Bindung, ein antibakterielles Mittel und ein Detergens umfasst.

29. Antigen-Verdünnungsmittel gemäß Anspruch 28, wobei das Puffermittel Natriumphosphat ist, der Chelatbildner EDTA ist, das Blockierungsmittel für nichtspezifische Bindung Gelatine ist, das chaotrope Mittel Natriumthiocyanat ist, das antibakterielle Mittel Natriumazid ist und das Detergens SDS ist.

30. Antigen-Verdünnungsmittel gemäß Anspruch 29, umfassend 25 mM Natriumphosphat, pH 6,5, 5 mM EDTA, 10 mM DTT, 0,2% Gelatine, 100 mM Ammoniumthiocyanat, 0,09% Natriumazid und 0,1% SDS.

31. Antigen-Verdünnungsmittel gemäß Anspruch 29, umfassend 50 mM Natriumphosphat, 5 mM EDTA, 100 mM Ammoniumthiocyanat, 0,06% SDS, 0,25% Fischgelatine und 10 mM DTT.

32. Antigen-Verdünnungsmittel gemäß Anspruch 1, wobei das Hepatitis C (HCV)-Antigen c33c, MEFA-6, c22p, c100p, NS-5 oder c200 ist.

33. Immunoassay-Kit für die Detektion von HCV-Antikörpern, wobei der Kit ein HCV-Antigen und ein Antigen-Verdünnungsmittel, umfassend ein Reduktionsmittel und ein chaotropes Mittel, umfasst.

34. Immunoassay-Kit gemäß Anspruch 33, wobei das Reduktionsmittel aus Dithiothreitol, Thioglycerin und Mercaptoethanol ausgewählt ist.

35. Immunoassay-Kit gemäß Anspruch 34, wobei das Reduktionsmittel Dithiothreitol ist.

36. Verwendung des Antigen-Verdünnungsmittels nach Anspruch 1 in einem Assay für die Detektion von Antikörpern, die gegen Hepatitis C-Virus-Antigene gerichtet sind.

Revendications

1. Diluant antigénique comprenant un antigène de l'hépatite C (HCV), un agent réducteur et un agent chaotropique.

2. Diluant antigénique selon la revendication 1, dans lequel l'agent réducteur est choisi parmi le dithiothréitol, le thioglycérol et le mercaptoéthanol.

3. Diluant antigénique selon la revendication 1, dans lequel l'agent réducteur est le dithiothréitol (DTT).

4. Diluant antigénique selon la revendication 3, dans lequel la concentration de DTT est de 1 mM à 200 mM.

5. Diluant antigénique selon la revendication 3, dans lequel la concentration de DTT est de 5 mM à 100 mM.

6. Diluant antigénique selon la revendication 3, dans lequel la concentration de DTT est d'environ 10 mM.

7. Diluant antigénique selon la revendication 1, comprenant en outre un agent tampon.

8. Diluant antigénique selon la revendication 7, dans lequel l'agent tampon est choisi parmi le phosphate de sodium ou le borate de sodium.

9. Diluant antigénique selon la revendication 8, dans lequel l'agent tampon est le phosphate de sodium.

10. Diluant antigénique selon la revendication 9, dans lequel la concentration de phosphate de sodium, pH 6,5, est de 15 mM à 100 mM.

11. Diluant antigénique selon la revendication 1, comprenant en outre un détergent.

12. Diluant antigénique selon la revendication 11, dans lequel le détergent est choisi parmi le dodécylsulfate de sodium (SDS) et le Tween-20®.

13. Diluant antigénique selon la revendication 12, dans lequel le détergent est le SDS.

14. Diluant antigénique selon la revendication 13, dans lequel la concentration de SDS est de 0,01 % à 0,5 %.

15. Diluant antigénique selon la revendication 1, comprenant en outre un agent antibactérien.

16. Diluant antigénique selon la revendication 15, dans lequel l'agent antibactérien est l'azoture de sodium.

17. Diluant antigénique selon la revendication 16, dans lequel la concentration d'azoture de sodium est de 0,01 % à 0,3 %.

18. Diluant antigénique selon la revendication 1, comprenant en outre un agent chélatant.

19. Diluant antigénique selon la revendication 18, dans lequel l'agent chélatant est l'acide éthylènediaminetétraacétique (EDTA).

20. Diluant antigénique selon la revendication 19, dans lequel la concentration d'EDTA est de 1 mM à 10 mM.

21. Diluant antigénique selon la revendication 1, comprenant en outre un agent bloquant les liaisons non spécifiques.

22. Diluant antigénique selon la revendication 21, dans lequel l'agent bloquant les liaisons non spécifiques est choisi parmi la gélatine et la sérumalbumine bovine.

23. Diluant antigénique selon la revendication 22, dans lequel l'agent bloquant les liaisons non spécifiques est la gélatine.

24. Diluant antigénique selon la revendication 23, dans lequel la concentration de gélatine est de 0,05 % à 1,0 %.

25. Diluant antigénique selon la revendication 1, dans lequel l'agent chaotropique est choisi parmi le thiocyanate de sodium et le thiocyanate d'ammonium.

26. Diluant antigénique selon la revendication 25, dans lequel l'agent chaotropique est le thiocyanate d'ammonium.

27. Diluant antigénique selon la revendication 26, dans lequel la concentration de thiocyanate d'ammonium est de 10 mM à 500 mM.

28. Diluant antigénique selon la revendication 1, comprenant en outre un agent tampon, un agent chélatant, un agent bloquant les liaisons non spécifiques, un agent antibactérien et un détergent.

29. Diluant antigénique selon la revendication 28, dans lequel ledit agent tampon est le phosphate de sodium, ledit agent chélatant est l'EDTA, ledit agent bloquant les liaisons non spécifiques est la gélatine, ledit agent chaotropique est le thiocyanate de sodium, ledit agent antibactérien est l'azoture de sodium et ledit détergent est le SDS.

30. Diluant antigénique selon la revendication 29, comprenant 25 mM de phosphate de sodium, pH 6,5, 5 mM d'EDTA, 10 mM de DTT, 0,2 % de gélatine, 100 mM de thiocyanate d'ammonium, 0,09 % d'azoture de sodium et 0,1 % de SDS.

31. Diluant antigénique selon la revendication 29, comprenant 50 mM de phosphate de sodium, 5 mM d'EDTA, 100 mM de thiocyanate d'ammonium, 0,06 % de SDS, 0,25 % de gélatine de poisson et 10 mM de DTT.

32. Diluant antigénique selon la revendication 1, dans lequel ledit antigène de l'hépatite C (HCV) est c33c, MEFA-6, c22p, c100p, NS-5 ou c200.

33. Kit d'immunoessai pour la détection d'anticorps anti-HCV, le kit comprenant un antigène de HCV et un diluant antigénique comprenant un agent réducteur et un agent chaotropique.

34. Kit d'immunoessai selon la revendication 33, dans lequel l'agent réducteur est choisi parmi le dithiothréitol, le thioglycérol et le mercaptoéthanol.

35. Kit d'immunoessai selon la revendication 34, dans lequel l'agent réducteur est le dithiothréitol.

36. Utilisation du diluant antigénique selon la revendication 1, dans un essai pour la détection d'anticorps dirigés contre les antigènes du virus de l'hépatite C.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 60059703 B **[0001]**
- WO 9300365 A **[0005]**
- WO 9641164 A **[0006]**
- WO 9632004 A **[0006]**
- US 5616460 A **[0006]**
- DE 19502386 **[0006]**
- WO 9425874 A **[0006]**
- JP 6074956 A **[0006]**
- EP 0341439 A **[0006]**
- US 9708950 B **[0029]**
- US 08859524 B **[0030]**
- WO 9527702 A **[0032] [0032]**

Non-patent literature cited in the description

- **SALLBERG.** *J. Clin. Microbiol.*, 1992, vol. 30, 1989-1994 **[0004]**
- **KOTWAL et al.** *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, 4486-4489 **[0005]**
- **CHIEN et al.** *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, 10011-10015 **[0006] [0028]**
- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual. 1989 **[0012]**
- DNA Cloning: A Practical Approach. vol. I,II **[0012]**
- Methods In Enzymology. Academic Press, Inc, **[0012]**
- Handbook of Experimental Immunology. Blackwell Scientific Publications, vol. I-IV **[0012]**
- Fundamental Virology. vol. I,II **[0012]**
- **KUO et al.** *Science*, 1989, vol. 244, 362-364 **[0028]**
- **COUSENS et al.** *Gene*, 1987, vol. 61, 265-275 **[0028]**
- **CHOO et al.** *Science*, 1989, vol. 244, 359-362 **[0028]**
- **CHIEN et al.** *Proc. Natl. Acad. Sci. USA*, 1989, vol. 89, 10011-10015 **[0028]**
- **EHELING et al.** *Lancet*, 1991, vol. 337, 912-913 **[0037]**